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Research and Development

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Project Summary

Effects of Ozone on Leukocyte DNA

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This research program was initiated with the overall objective of determining whether exposure to ozone could damage the DNA of peripheral blood cells.

An animal model system was designed in which glycogen was used to stimulate the production of peritoneal exudate cells (PECs) in mice. These PECs were labeled by repeated i.v. injection of ^{3}H-thymidine. The labeled PECs circulated briefly through the peripheral blood and eventually accumulated in the peritoneal cavity. The experimental animals were either exposed to ozone or ambient atmosphere (sham treated) during the period of time when the PECs were circulating through the peripheral blood. These PECs were then harvested and their DNA analyzed for single strand breaks on alkaline sucrose gradients.

At very high levels of ozone exposure (5 ppm 9800 $\mu g/m^3$ 24 hours) DNA damage was readily apparent. At lower levels of ozone (1 ppm for 24 hours), some DNA damage may have occurred. In each experiment at this level, some decrease in the weight average molecular weight of the DNA from ozone exposed animals was detected. However, the data were not statistically significant in terms of increased number of single strand breaks. No attempt was made to assess DNA damage at even lower ozone levels.

This project raises the possibility that some damage may occur to the DNA of peripheral blood cells during exposure to environmentally realistic levels of ozone. Unfortunately, the currently available technology for assaying DNA damage is not sufficiently sensitive to unambiguously resolve this question.

This Project Summary was developed by EPA's Health Effects Research Laboratory, Research Triangle Park, NC, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

In addition to its direct effects on lung tissue, ozone has been shown to cause a number of systemic effects such as changes in visual acuity, sphering of red blood cells, and alterations in serum enzyme levels. Many of these systemic effects are radiomimetic. That is, they appear to mimic the type of damage caused by ionizing radiation, and thus are assumed to be caused by the generation of free radicals within the target cell.

This project dealt with one such example of systemic damage. Previous investigators have observed that ozone causes chromosome aberrations in peripheral blood lymphocytes of both Chinese hamsters and human KB cells. This implies, of course, that ozone causes considerable DNA damage in these cells. If this is true, it provides an extremely important avenue of research into the mechanism of ozone toxicology which has yet to be followed up.

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Another unresolved question is the manner in which ozone caused systemic damage. Ozone itself is very reactive and might be expected to react primarily with the lung tissue without penetrating any further. It is widely assumed, therefore, that ozone does not cause systemic damage directly, but via production of peroxides andozonides which migrate to the target cell and generate free radicals. For the most part, this mechanism is speculative, although it has been shown that ozonides and peroxides are produced in response to ozone exposure and that they can cause some of the observed effects in cell culture.

One method of assessing ozone related damage to the DNA is to measure the extent of DNA repair synthesis. Cultured lymphocytes have previously been shown to carry out repair of damage to their DNA caused by UV radiation, ionizing radiation, or alkylating agents. This repair DNA synthesis is quite specific and easy to measure. In addition, the magnitude of the repair synthesis is proportional to the extent of the damage. Lymphocytes, in fact would appear to provide an ideal system for studying DNA repair, since normal replicative DNA synthesis is quite low in unstimulated lymphocytes.

Since most examples of systemic ozone damage do seem to mimic the effects of ionizing radiation and since ozone apparently does damage lymphocyte DNA, one would predict that exposure to ozone should set off a round of repair synthesis in the circulating blood lymphocytes of the exposed animal. If this prediction held true, it would provide a valuable new probe for measuring the extent and mechanism of ozone damage. In the first place, the magnitude of the repair synthesis should be proportional to the extent of damage. Secondly, by examining the repair process closely, one should be able to gather some information as to the mechanism by which ozone damaged the DNA. In this project, the extent of DNA repair synthesis in cultured lymphocytes was measured following exposure of rabbits, guinea pigs, and hamsters to ozone.

Of course, since ozone damage should be radiomimetic, one would expect the damaged DNA to contain an increased number of single strand breaks. Thus, it should also be possible to measure the effects of ozone on the DNA directly by looking for single strand breaks in the DNA. In this project the number of single strand breaks in the DNA of peritoneal exudate cells was measured by alkaline elution profile or by alkaline sucrose gradient following exposure of mice to ozone.

Conclusions

This assay technique does not appear to be sensitive enough to detect DNA damage by the exposure of the whole animal to ozone. Clearly, exposure of the mice to 5 ppm (9800 $\mu g/m^3$) ozone for 24 hours causes a significant increase in single strand breaks in the DNA of the leukocytes which are formed and which circulate through the peripheral blood during the ozone exposure. While this experimental result establishes the validity of the experimental approach, it does not provide any information on the chances of DNA damage occurring upon exposure to levels of ozone which are likely to be found in the environment. In the first place, exposure to 5 ppm ozone for a 24-hour period is extremely unlikely. Secondly, previous experiments have shown that normal defense mechanisms begin to be overwhelmed at 5 ppm.

While exposure to 1 ppm ozone for a 24-hour period is unlikely, exposure to levels approaching 1 ppm for shorter times does occur. In these experiments a 24-hour exposure to 1 ppm did appear to cause a slight decrease in the weight average molecular weight of the DNA in each of the four separate experimental trials. This suggests that some DNA damage in the form of single strand breaks was occurring. However, the difference in size distribution was rarely statistically significant. Thus, these experiments suggest that some DNA damage may occur at lower levels of ozone exposure, but the technique is not sensitive enough to accurately determine whether damage occurs at environmentally significant doses.

Finally, the effect of 48-hours exposure to 1 ppm (1960 $\mu g/m^3$) was measured in a single experiment. In this case, no decrease in weight average molecular weight of the DNA was noted following ozone exposure. The reasons for this could be twofold: 1) Most of the leukocyte accumulation in the peritoneal cavity will have occurred by 24 hours. By 48 hours, a repair synthesis may have ligated the damaged DNA. 2) By 48 hours, the cell population which accumulates in the peritoneal cavity is more heterogeneous. The other cell types which accumulate may be less sensitive to ozone-caused DNA damage.

Recommendation

This study still leaves open the possibility that ozone may damage the DNA of peripheral blood cells. As more sensitive techniques for assaying DNA damage become available, this question should be reinvestigated.

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***Gary Hatch** is the EPA Project Officer (see below).*
The complete report, entitled "Effects of Ozone on Leukocyte DNS," (Order No. PB 81-179 277; Cost: $6.50, subject to change) will be available only from:
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Telephone: 703-487-4650
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